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CHOLESTEROL-LOWERING PEPTIDES

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Title of the Invention: Cholesterol-lowering peptides.

Summary

Means to solve: Cholesterol-lowering peptides L and I with the following amino acid sequences: Peptide L: Leu-Asp-Ile-Gln-Lys. Peptide I: Ile-Ile-Ala-Glu-Lys.

Benefits: With these, safe and highily efficient cholesterol-lowering drugs and beverages can be made.

Claims

(1) The cholesterol-lowering peptide, peptide L, with the amino acid sequence No. 1 shown in the list.
(2) Cholesterol-lowering peptide, Peptide I, of the amino acid sequqence No. 2 shown in the list.
(3) Cholesterol-lowering agents with peptide L and/or peptide I as the effective ingredient.
(4) Cholesterol-lowering baverages containing peptide L and/or peptide I.

Technical field of the invention

This invention is about the lowering of cholesterol. To be more specific, it is about the cholesterol-lowering system(s) using new cholesterol-lowering peptide(s). The peptides mentioned in the invention can not only used in medicine as the cholesterol-lowering agents but also in other wide areas such as in beverages, foods, functional foods, nutritional foods and health foods to prevent accumulation of cholesterol.

Previous technics

Cholesterol and its derivatives are essential component of the body. Triacylglycerols serve only to store energy while cholesterol and its fatty acid esters areimportent structural components of cytoplasmic membranes but also produce various derivatives with bile acids, steroid hormones and vitamin D, etc. to perform various key functions. Control of cholesterol metabolism is important in the maintenance of health of cardio-vascular system while its abnormality bring artherosclerosis.

Lately lots of reasearches have been carried out throughout the world on the dietary fibers, soy proteins and unsaturated fatty acids on their good effects on cholesterol metabolism. For example, Carrol and Hamilton studied the effects of dietary proteins on plasma cholesterol and demonstrated that soy protein can lower plasma cholesterol more than casin protein [K.K. Carrol and R.M. G. Hamilton, J. Food Sci., 40: 18-23 (1975)]. Also, according to Japanese Patent Publication Sho 60-11425, oligopeptides of the molecular weight of 200 to 1500, obtained from enzymatic hydrolysis of soy protein can lower cholesterol concentration in blood. Thus, plant proteins typified by soy protein and their enzymic hydrolysates seems generally able to lower plasma cholesterol and prevent artherosclerosis.

Problems that this invention intends to solve

As mentioned above, in view of the technical situations of active research and development now under way on the plasma cholesterol lowering agents derived from soy proteins the inventors aimed to do some research on completely new type of peptides not derived from soy proteins which are safe and highly effective in the lowering of cholesterol.

Means to solve the problems

To solve the said problems the inventors studied the subject from various angles. They focused on peptides, particularly oligopeptides and synthesized many peptides and screening. They found two peptides (peptides L and I) which have excellent plasma cholesterol-lowering effects. Further studies showed that they could be used in chlocsterol-lowering agents and beverages and thus came up with the invention.

The peptides L and I consist of amino acid sequences No. 1 and 2 which are shown below:
Peptide L: Leu-Asp-Ile-Gln-Lys. Peptide I: Ile-Ile-Ala-Glu-Lys.

These peptides have never been synthesized nor isolated and their cholesterol-lowering effects have never been known but they can readily synthesized or obtained from automatic peptide synthesizer, etc.

As is clear from the examples to be mentioned later, these peptides can significantly reduce cholesterol level so other than using them in various kinds of food and beverages like specific health drinks, health foods, health drinks, nutritional foods and other types of foods and drinks (drinks are included in foods in this invention), they can be used as cholesterol absorption inhibitors and cholesterol-lowering drugs, etc.

When used in foods and drinks the peptide(s), the effective ingredient(s), are used as such or used together with other food ingredients. The foods and drinks using the said effective ingredients of the invention can be solids (powder, granules and others), pastes, liquid or suspension but it is ideal to use health drinks together with sweetners, vitamins and acidifying agents, etc. those ingredients commonly used in the drinks.

When used as medicine the said effective ingredients can be added in various forms. For example, they can be made into oral drugs as tablets, capsules, granules, powders and syrup, etc. These drugs can be prepared by the ordinary procedures together with the use of binder, lubricant, disintegrator, diluent base, solution aid, suspension aid, coating agent, taste correcting agent, etc. The dose depends on the symptoms, age, body weight, administration route and shape of the drug, etc. but usually about 0.1 mg to 1,500 mg is given per day to an adult.

The effective ingredients of this invention are either completely free of toxicity or with very low toxicity so they are very safe. No acute toxicity was noticed at all to rats when 500 mg was orally given per day. Therefore, when used as beverages there is no specific limitation on the dose either for preventive, health or drinking purpose. Even when used as medicine suitable dose can be given depending on the patients. Also, the effective ingredients of this invention do not show any specific acute toxicity when used in large dose so if necessary, they can be given more than the range specified above.

In the following sections examples will be given to describe the invention more in detail.

Example 1:

Peptides L and I were prepared with an automatic peptide synthesizer. Purity of the resultant peptides was confirmed to be 95% by reverse phase HPLC. The cholesterol-lowering effects of these peptides was verified by the in vivo test using rats.

Wistar male rats (three weeks old, initial body weight 30 gm) were fed with feed containing 20% casein, 1.0% cholesterol and 0.25% sodium cholate for four days. Composition of

for about three hours while maintaining pH, then the solution was cooled rapidly, and after adjusting pH to 6.9-7.0 the enzymes was inactivated with a plate type sterilizer. By atomizing the solution about 9.0 Kg of beta-lactoglobulin trypsin hydrolysate was obtained.

Twentyfour hours after the third day oral administration rats were sacrificed by cardiac blood collection. Serum was prepared by centrifuging blood at 3,000 rpm for 15 minutes. Assay of serum cholesterol was done enzymatically, i.e., using the commercial kit (monotest cholesterol, Behringer-Manheim Yamanouchi Kabushiki Kaisha). The standard was pretiset cholesterol, Behringer-Manheim Yamanouchi Kabushiki Kaisha. Likewise HDL-cholesterol was determined with HDL-cholestase (Nissui Seiyaku Kabushiki Kaisha). LDL + VLDL-cholesterol was calculated. Results are shown in Tables 2, 3 and 4.

Table 2

Results (1)

	C	Beta
(a)	529.31 ± 14.37d	435.38 ± 12.82c***
(b)	42.61 ± 1.62a	52.15 ± 1.70b**
(c)	486.70 ± 26.74c	383.24 ± 24.16b*
(b)/(a)	0.08 ± 0.01a	0.12 ± 0.01b**

Table 3

Results (2)

	G	L
(a)	441.33 ± 11.97c***	392.49 ± 9.50b***
(b)	50.52 ± 2.41b*	47.82 ± 1.87ab
(c)	390.81 ± 22.91b*	344.66 ± 18.14ab**
(b)/(a)	0.12 ± 0.01b*	0.12 ± 0.01b**

Table 4

Results (3)

	I
(a)	328.70 ± 11.28a***
(b)	47.48 ± 0.82ab*
(c)	281.22 ± 20.59a***
(b)/(a)	0.15 ± 0.01c***

In the tables (a): serum cholesterol (mg/dl); (b) HDL-cholesterol (mg/dl); (c) LDL + VLDL-cholesterol (mg/dl); (b)/(a): Atherogenic index. Each figure was the mean ± SEM of seven animals per group. In statistical analysis Duncun's multiple range test and Student's t-test were used. In addition, in the tables *p 0.05, **p 0.01 and ****p 0.001.

From the said results it was verified that both peptides L and I had excellent cholesterol-

the said feed is shown below (Table 1).

Table 1 (gm/1.2 Kg)

	(%)	1C
Casin	20	279.07
Lard	5	60
Corn oil	1	12
Cellulose	5	60
Mineral 1	3.5	42
Vitamin 2	1	12
Sucrose		239.18
Starch		478.35
Cholesterol	1	12
Sodium cholate	0.25	3
Choline chloride	0.2	2.4
Total		1200.00

Note: 1C: 20% casein + 1% cholesterol. Sucrose and starch were mixed at 1:2 ratio.

In the above mineral a was a AIN-76 mineral mixture and the said feed contained the following manounts of the minerals (mg) per Kg of feed.

Mineral mixture. AIN-76 mineral mixture (mg/Kg): Ca, 5200; P, 4000; K, 3600; Na, 1020; Mg, 500; Mn, 54; Fe, 35; Cu, 6; Zn, 30;, I, 0.2; Se, 0.2; Cr, 2.0;, Cl, 1560; sulfate, 1000.

In the above table vitamin 2 was a AIN-76 vitamin mixture and the said feed contained the following vitamins (mg) per Kg of feed.

Vitamin mixture. AIN-76 vitamin mixture (mg/Kg): Thiamine-HCl, 6.0; riboflavin, 6.0; pyridoxine-HCl, 7.0; nicotinic acid, 30; calcium panthothenate, 16; folic acid, 2.0; biotin, 0.2; menadione, 0.05; lecinyl palmitate, 2.67 (4,000 IU), ergocalciferol, 0.025 (10, 000 IU), di-alpha-tocopherol-HCl, 100 (100 IU).

Rats were dividied into five groups (groups C, beta, G, L and I. Each group with seven rats). Groups C (casein trypsine hydrolysate), beta (beta-cystosterol), C (beta-lactoglobulin trypsine hydrolysate), L (synthetic peptide L) and I (synthetic peptide I) were each given 200 mg/Kg/day at 8.00 o-clock on days 2 and 3. In addition, the betal-lactoglobulin trypsine hydrolysate given to rats in group G was prepared as follows, so the casein trypsin hydrolysate for group C.

Ten Kg of beta-lactoglobulin, isolated from sweet whey was dissolved in 190 Kg of water. Temperature of the solution was kept at 42 °C. Sodium hydroxide was added to make pH of the solution 7.5-8.0. One hundred gm of trypsin (NOVO Co. 4500K) previously dissolved in 0.01 NH_4CL solution was added to the said solution. Enzymic hydrolysis was carried out

lowering effects.

Example 2:

To the mixture consisting of 20 gm of vitamin C, 50 gm of granular sugar and 30 gm of equal weight mixture of corn starch and lactose was added 50 gm of peptide L for good mixing. The mixture was dividied into 100 equal parts and put into bags so each bag contained 1.5 gm of stick-like cholesterol-reducing nutritional health food.

Example 3:

Preparation was done as in Example 2 exept that instead of 50 gm of peptide L, 40 gm of peptide I was used. Also, instead of 20 gm of vitamin C 30 gm of equal mixture of vitamin C and citric acid was used. Cholesterol-reducing nutritional health food was prepared after through drying.

Example 4:

One hundred gm of peptide L powder, 150 gm of sugar, 15 gm of bee honey, one gm of ascorbic acid, 0.5 gm of citric acid and suitable amount of fragrant material were added together with water to make it one Kg. The mixture was sterilized at 95 °C for 20 minutes and 100 ml each was filled aseptically into each bottle to make health drinks.

Example 5:

Two hundred gm of the 20% aqueous solution of peptide I powder, 5 gm of acetic acid tocophoerol, 10 gm of thiamine nitrate, 20 gm of nicotinamide, 50 gm of anhydrous caffeine, suitable amounts of benazoate and fragrant material and deionized water were mixed to the final volume of 30 liters. After sterilization 30 ml each was filled into the bottles aseptically to make health drink.

Example 6:

(1) Peptide L---- 50 gm. (2) lactose ----- 90 gm. (3) Corn starch ---- 29 gm. (4) Magesium stearate ---- 1 gm.

(1), (2) and (3) (but 17 gm) were mixed, and together with the paste prepared from (3) (but 7 gm) they were pelletized. To the pellets (3) (but 5 gm) and (4) were added and well-mixed and the mixture was compressed in a compression tablet machine to make 1000

pieces of tablets containing 50 mg of the effective ingredient (L) per tablet.

Example 7:

Tablets were made as in Example 6 except that peptide I was used instead of peptide L.

Dose depends on the conditions of the patients and their age but generally it is 0.1-1500 mg/Kg/day and 1-4 times a day. The peptides used in the invention are almost completely safe as described above so higher dose can also be used. Also, when used to maintain, promote health or as nutrient they can be given at doses slightly less than that mentioned above for long period of time.

Advantages

According to this invention the peptides can effectively lower werum cholesterol level and very safe so they can not only used to prevent or treat for serum cholesterol reduction. Besides, they do not have unpleasant taste like strong bitterness so they can be used in drinks to prevent or treat for cholesterol reduction.